Environmental Health Perspectives
Vol. 71, pp. 25-29, 1987

Nerve Membrane Ion Channels as the Target Site of Environmental Toxicants

by Toshio Narahashi*

There are many environmentally important chemicals which exhibit potent effects on the nervous system. Examples include insecticides such as pyrethroids, DDT, cyclodienes, organophosphates and carbamates, and heavy metals such as mercury and lead. Since nerve excitation takes place in a fraction of a second, electrophysiological methods provide us with the most straightforward approach to the study of the mechanisms of action of environmental toxicants on the nervous system. Aquatic animals such as crayfish, lobster, squid, and marine snails represent extremely useful materials for such electrophysiological studies, because much of our knowledge of nerve excitation is derived from those animals.

Nerve excitation takes place as a result of opening and closing of ion channels of the membrane. These functions are independent of metabolic energy, and can be measured most effectively by voltage clamp techniques as applied to the giant axons of the crayfish and the squid. Patch clamp techniques developed during the past 10 years have added a new dimension to the electrophysiological investigation. These techniques allow us to measure the activity of individual ion channels, thereby making it possible to analyze the interaction of toxic molecules directly with single ion channels.

Examples are given summarizing electrophysiological studies of environmental neurotoxicants. The abdominal nerve cords and neuromuscular preparations isolated from the crayfish are convenient materials for bioassay of certain environmental toxicants such as pyrethroids, chlorinated hydrocarbons, and other insecticides. Detailed voltage clamp and patch clamp analyses have revealed that pyrethroids and DDT modify the sodium channel to remain open for an extended period of time. This change causes an increase in depolarizing afterpotential which reaches the threshold for repetitive discharges to be produced. The latter is the basis for severe symptoms of poisoning in animals. Only a small fraction of the flux through the sodium channel, less than 1%, must be modified by pyrethroids for the animal to develop symptoms of poisoning. Such a toxicological amplification from channel to animal is important in understanding the potent toxic effect.

Introduction

Numerous environmental toxicants are known to cause serious damage to the nervous system. These neurotoxic substances include insecticides, heavy metals, and hexanes. Indeed, most of the insecticides currently in use for agricultural, medical, and veterinary purposes are very potent neuropoisons as exemplified by pyrethroids, DDT, cyclodienes, organophosphates and carbamates. The environmental hazards of heavy metals such as mercury and lead are due primarily to damage, both acute and chronic, to the nervous system. Therefore, to prevent and manage intoxication by environmental neurotoxicants, it is imperative to understand the mechanisms of toxic action of these agents on the nervous system.

Various approaches and methods have been used to accomplish this goal, including electrophysiological, neurochemical, histological, and behavioral techniques. Since the major function of the nervous system is to generate and transmit excitation in the form of an electrical signal, or impulse, electrophysiological techniques provide us with the most straighforward and powerful approach to elucidate the mechanisms of action of neurotoxicants at the cellular and molecular level.

Aquatic animals represent an important group with respect to environmental neurotoxicology for several reasons. First, some of them provide us with very unique and important materials for the study of the mechanism of action of neurotoxicants. For example, the nervous systems of the crayfish, lobster, squid, and marine snails have been used very widely to study the mechanisms of nerve excitation in general. Almost all aspects of our present knowledge of nerve excitation are derived from study of aquatic animal models. These nerve preparations have been used extensively for the study of neurotoxicants as well. Second, because aquatic animals are very sensitive to certain environmental neurotoxicants such as cyclodienes, they are important for gaining an understanding of differential sensitivity.

This paper summarizes some of our recent environmental studies on aquatic animals. Major emphasis is on the study of neuroactive insecticides on the nervous system of the crayfish and squid. Similar approaches can be easily applied to the study of other environmental

*Department of Pharmacology, Northwestern University Medical School, 303 East Chicago Avenue, Chicago, IL 60611

neurotoxicants. It is important to realize that information gained through these studies utilizing aquatic animals can be easily applied to the human. Therefore, such studies can contribute immensely to our health care with respect to the environmental toxicants.

Mechanisms of Nerve Excitation

The nerve membrane generates impulses or action potentials which are transmitted from the sensory cells to the central nervous system and then to the motor system. The action potential is generated as a result of changes in membrane permeabilities to ions such as sodium, potassium and calcium. The resting membrane potential (RP), inside negative with respect to the outside by 50 to 100 mV, assumes a value close to the equilibrium potential for potassium (E_K), because the resting membrane is almost exclusively permeable to potassium (Fig. 1). When the membrane is depolarized (stimulated), the membrane permeability (conductance) to sodium (g_{Na}) increases rapidly, so that the membrane potential approaches a value close to the equilibrium potential for sodium (E_{Na}); this is the rising phase of the action potential (AP). However, the increased g_{Na} starts decreasing quickly and at about the same time the potassium permeability (conductance) g_K starts increasing beyond its resting value. These changes in g_{Na} and g_K bring the membrane potential back toward the potassium equilibrium potential; this is the falling phase of the action potential.

During the rising phase of the action potential, sodium ions enter the cell according to their electrochemical gradient, and during the falling phase of the action potential, potassium ions leave the cell according to their electrochemical gradient. The increases in g_{Na} and g_K are the result of opening of "sodium channels" and "potassium channels," respectively, so that the sodium influx and potassium efflux occur through these open channels. The resultant increase in intracellular sodium concentration and decrease in intracellular potassium concentration are very small indeed, being calculated to be approximately 1/1000 of their initial concentrations for a nerve fiber 1 μm in diameter.

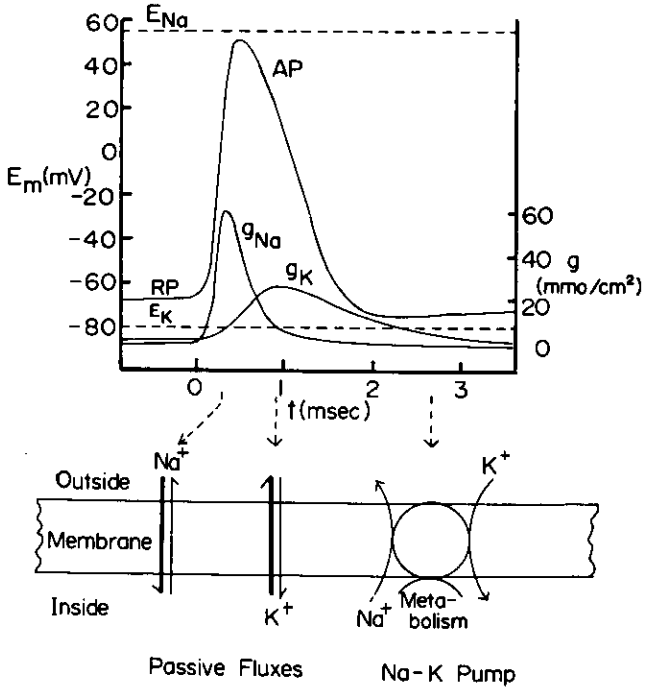

FIGURE 1. Diagram of the mechanism of resting and action potential production. RP, resting potential; AP, action potential; E_K, potassium equilibrium potential; E_{Na}, sodium equilibrium potential; g_{Na}, sodium conductance; g_K, potassium conductance (*4*).

However, no matter how small the internal ionic concentration changes may be, they must be restored in order for the nerve fiber to continue to generate many action potentials. This is accomplished by a mechanism called the Na–K pump, which pushes out extra sodium and absorbs potassium. This pump is operated by metabolic energy. By contrast, changes in g_{Na} and g_K, or opening and closing of sodium and potassium channels, take place normally in the absence of metabolic energy, and therefore are metabolism-independent processes.

When an action potential arrives at the nerve terminal, a neurotransmitter is released. The transmitter in turn binds to the postsynaptic receptor, resulting in changes in ionic permeabilities in the postsynaptic membrane. These changes in permeabilities generate action potentials in the postsynaptic neurons or effector cells, thus completing synaptic transmission. A large number of neurotransmitters have been identified in various animals and synapses, including acetylcholine, norepinephrine, glycine, glutamate, γ-aminobutyric acid, and certain peptides, to mention a few. Postsynaptic ionic permeabilities are due to various ion channels permeable to sodium, potassium, calcium, chloride, etc., depending on the synapse.

Electrophysiological Methods

Routine electrophysiological methods utilizing extracellular electrodes or intracellular microelectrodes are useful for recording action potentials from nerve fibers, sensory neurons, and synapses. However, these methods are far from sufficient to elucidate the mechanism of action of neurotoxicants on membranes, as they do not permit analysis in terms of ion channels. The function of ion channels can best be studied by voltage clamp techniques, which allow us to measure ionic permeabilities in the form of ionic conductances. Voltage clamp techniques were originally developed for the squid giant axons by Cole (*1*) and improved and extensively used by Hodgkin, Huxley, and Katz (*2*). It has now become a routine technique for the study of neurotoxicant effects on nerve membrane ion channels (*3,4*). The voltage clamp technique was also adapted to postsynaptic membranes such as end-plate membranes (*5*).

A dramatic advance in technology was made about 10 years ago by Neher and Sakmann (*6*), who successfully developed a patch clamp technique to record opening and closing of individual ion channels. The technique

has been much improved in the interim, and now it is possible to measure single channel activity of practically any cell, including inexcitable cells (*7*). We can now analyze interactions of toxic molecules with a single ion channel. Much progress has been made for the study of environmental neurotoxicants also (*8–10*).

Crayfish Nerve Cords

The abdominal nerve cord isolated from the crayfish is a convenient and sensitive material for simple bioassay of certain neurotoxicants (*11*). Spontaneous discharges, as recorded by a pair of wire electrodes, can serve as a measure of either stimulating or paralyzing effects of neurotoxicants. An example of an experiment for the pyrethroid allethrin is shown in Figure 2. Allethrin at a concentration of 1 μM causes a drastic increase in spontaneous discharges within 5 min, followed by complete paralysis later. Even at a lower concentration of 100 nM, allethrin causes a large increase in the frequency of spontaneous discharges (Fig. 3). Other insecticides such as carbofuran, DDT, and toxaphene have also been found to stimulate the nerve cord.

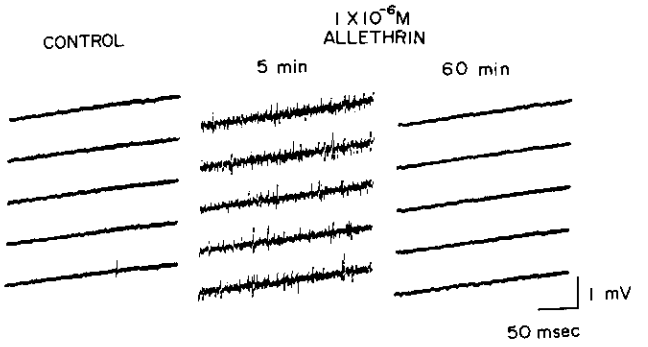

FIGURE 2. *Effects of 1 μM allethrin on the spontaneous discharges of the crayfish abdominal nerve cord (11).*

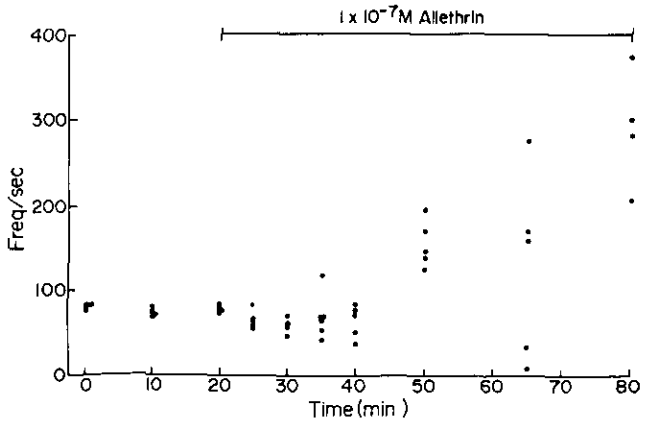

FIGURE 3. Effects of 100 nM allethrin on the frequency of spontaneous discharges of the crayfish abdominal cord. Each point represents a measurement during a period of 0.2 or 1 sec.

Crayfish Neuromuscular Preparations

Crayfish neuromuscular preparations have also been found to be a very useful material for bioassay of certain toxicants. These preparations have both excitatory and inhibitory innervation. L-Glutamate and γ-aminobutyric acid are the respective transmitters. Excitatory transmission is very sensitive to pyrethroid and DDT-type compounds. An example is illustrated in Figure 4 in which muscle contractions evoked by nerve stimulation are first augmented and then blocked by 100 nM allethrin. It was later found that the augmented contraction produced by pyrethroids and DDT-type insecticides was due primarily to the stimulation of presynaptic nerves (*12*). Repetitive discharges were generated in the presynaptic nerve which caused repetitive and augmented activity of the muscle.

Crayfish and Squid Giant Axons

The giant axons isolated from the crayfish or the squid have proved to be among the most convenient materials to determine the mechanisms of action of neurotoxicants. First, the basic mechanism of nerve excitation has been studied extensively using these preparations. In fact, because of its large size the squid giant axon is the prototype nerve preparation from which most of our present knowledge of nerve excitation has been derived. Second, voltage clamp experiments to analyze the ion channel function can be performed with these giant axons with the highest degree of accuracy. Third, these giant axons can be perfused intracellularly as well as extracellularly, providing us with a high degree of flexibility for voltage clamp measurements. Fourth, the materials are relatively readily available.

In order to illustrate the usefulness of these giant axon preparations and to summarize our current knowledge of the mechanisms of action of toxicants, some of our studies of DDT and pyrethroid insecticides will be briefly described. When applied to the isolated giant axon, some of these insecticides cause repetitive afterdischarges in response to a single stimulus (Fig. 5). Repetitive afterdischarges are produced when the depolarizing afterpotential is elevated to the level of threshold for excitation. Thus, the next question is how the depolarizing afterpotential is increased by DDT or pyrethroids. This can best be studied by voltage clamp experiments.

Voltage clamp experiments with crayfish and squid giant axons have clearly shown that the sodium current is greatly prolonged by pyrethroids and DDT (*13–16*). An example of such an experiment is shown in Figure 6. The control record represents the transient sodium current as evoked by a step depolarizing pulse. After application of allethrin, the sodium current is greatly prolonged while its peak amplitude remains unchanged. A prolonged sodium current increases the depolarizing afterpotential, which in turn generates repetitive afterdischarges. Thus, the major effect of pyrethroids and DDT is to prolong the sodium current.

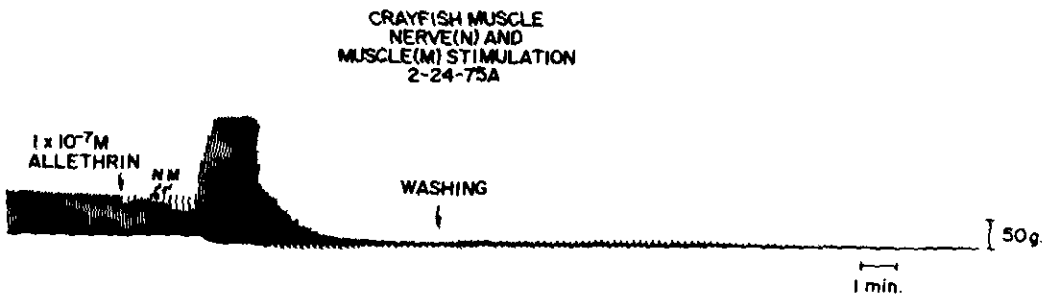

FIGURE 4. Effects of 100 nM allethrin on the contractions of the crayfish muscle evoked by nerve (N) and muscle (M) stimulations (*11*).

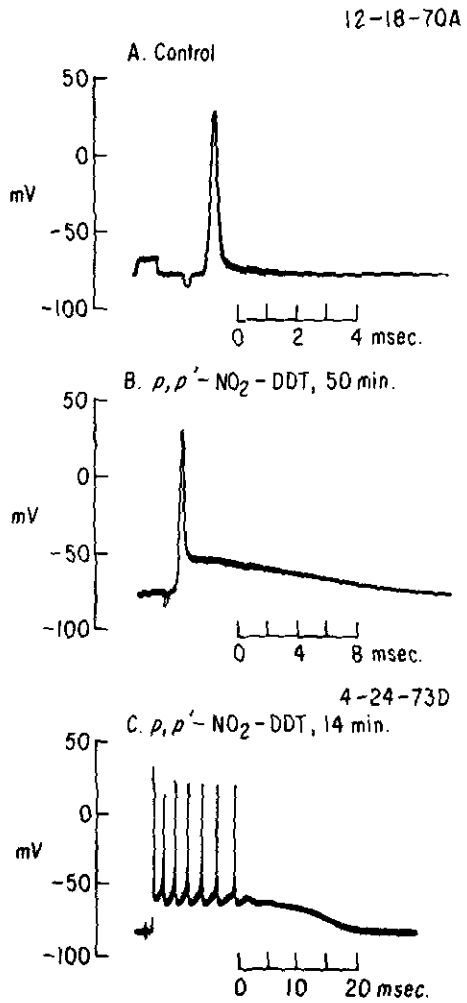

FIGURE 5. Effects of *p,p'*-NO_2-DDT, an analog of DDT, on the action potential recorded intracellularly from an isolated crayfish giant axon: (A) control; (B) 50 min after application of 50 μM NO_2-DDT; (C) 15 min after application of 100 μM NO_2-DDT (*18*).

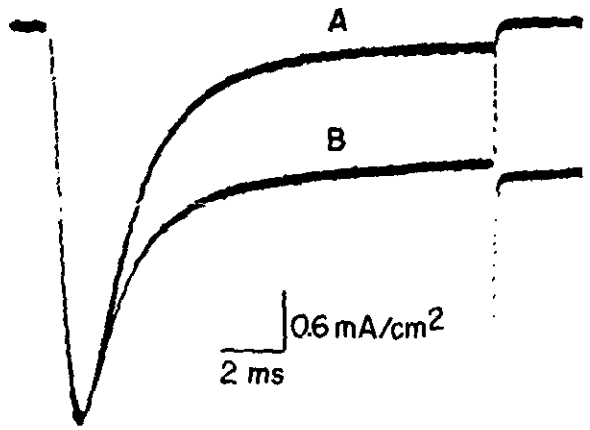

FIGURE 6. Effects of 1 μM allethrin on the sodium current recorded from a squid giant axon under voltage clamp conditions: (A) control, (B) allethrin (*4*).

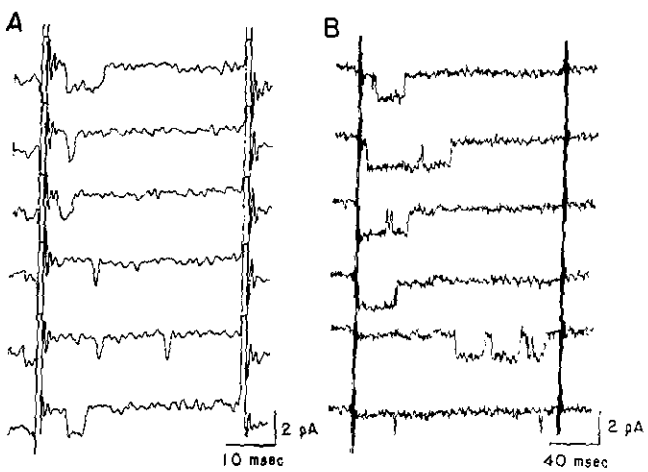

FIGURE 7. Single sodium channel currents of the membrane of a neuroblastoma cell (*A*) before and (*B*) after application of 60 μM (+)-*trans*-tetramethrin (*10*).

Single Channel Experiments

The sodium current recorded from a giant axon under voltage clamp conditions is a sum of individual sodium channel currents. Thus, it is difficult to analyze direct interactions of insecticide molecules with individual sodium channels from the classical voltage clamp experiments with giant axons. Single channel recording techniques, i.e., patch clamp, provide us with an excellent opportunity to study this aspect. It has indeed been

shown that pyrethroids cause a remarkable prolongation of sodium channel opening (*8–10*).

An example of a single channel experiment is illustrated in Figure 7. Single sodium channel currents were recorded from a neuroblastoma cell (N1E-115 line) by using the patch clamp technique (*10*). In the control cell, individual sodium channels open during a step depolarizing pulse and can be seen as downward square deflections on the record. After application of 60 μM tetramethrin, individual sodium channel currents are still observed with their amplitude unchanged. However, individual channels are kept open for a much longer period of time. Analyses of the open time indicate that in the presence of tetramethrin there are two populations of sodium channels, one with the normal open time and the other with a prolonged open time. The latter represents the sodium channels modified by tetramethrin. Thus, tetramethrin modifies the individual sodium, channels in an all-or-none manner.

From Channel to Animal: Toxicological Amplification

The observed change by pyrethroids at the single sodium channel level can account for the symptoms of poisoning at the animal level. Calculations were made of the percentage of sodium channel population that must be modified by tetramethrin for the depolarizing afterpotential to reach the threshold for repetitive discharges. Only a very small fraction of sodium channels, less than 1%, needs to be modified for this change in depolarizing afterpotential which leads to severe symptoms of poisoning in animals (*17*). This is why the pyrethroids are very potent as insecticides. This situation also provides us with an excellent example of "toxicological amplification" from ion channel to animal.

Our studies cited in this paper were supported by NIH grant NS14143. I thank S. Collins for secretarial assistance.

REFERENCES

1. Cole, K. S. Dynamic electrical characteristics of the squid axon membrane. Arch. Sci. Physiol. 3: 253–258 (1949).
2. Hodgkin, A. L., Huxley, A. F., and Katz, B. Measurement of current-voltage relations in the membrane of the giant axon of *Loligo*. J. Physiol. 116: 424–448 (1952).
3. Narahashi, T. Pharmacology of nerve membrane sodium channels. In: Current Topics in Membranes and Transport, Vol. 22, The Squid Axon (P. F. Baker, Ed.), Academic Press, New York, 1984, pp. 483–516.
4. Narahashi, T. Drug-ionic channel interactions: single channel measurements. In: Basic Mechanisms of the Epilepsies (Annals of Neurology, Vol. 16, Supplement), Little, Brown and Company, Boston, MA, 1984, pp. S39–S51.
5. Takeuchi, A., and Takeuchi, N. Active phase of frog's end-plate potential. J. Neurophysiol. 22: 395–411 (1959).
6. Neher, E., and Sakmann, B. Single-channel currents recorded from membrane of denervated frog muscle fibres. Nature 260: 779–802 (1976).
7. Sakmann, B., and Neher, E. (Eds.). Single-Channel Recording. Plenum Press, New York, 1983.
8. Chinn, K., and Narahashi, T. Stabilization of sodium channel states by deltamethrin in mouse neuroblastoma cells. J. Physiol. 380: 191–207 (1986).
9. Holloway, S. F., Salgado, V. L., Wu, C. H. and Narahashi, T. Maintained opening of single Na channels by fenvalerate. 14th Ann. Meeting Soc. Neurosci. Abstr. Vol. 10, 1984, p. 864.
10. Yamamoto, D., Quandt, F. N., and Narahashi, T. Modification of single sodium channels by the insecticide tetramethrin. Brain Res. 274: 344–349 (1983).
11. Takeno, K., Nishimura, K., Parmentier, J., and Narahashi, T. Insecticide screening with isolated nerve preparations for structure-activity relationships. Pestic. Biochem. Physiol. 7: 486–499 (1977).
12. Farley, J. M., Narahashi, T., and Holan, G. The mechanism of action of a DDT analog on the crayfish neuromuscular junction. Neurotoxicology 1: 191–207 (1979).
13. Narahashi, T. Mode of action of chlorinated hydrocarbon pesticides on the nervous system. In: Halogenated Hydrocarbons: Health and Ecological Effects (M. A. Q. Khan, Ed.), Pergamon Press, Elmsford, NY, 1981, pp. 222–242.
14. Narahashi, T. Nerve membrane sodium channels as the target of pyrethroids. In: Cellular and Molecular Neurotoxicology (T. Narahashi, Ed.), Raven Press, New York, 1984, pp. 85–108.
15. Narahashi, T. Nerve membrane ionic channels as the primary target of pyrethroids. Neurotoxicology 6: 3–22 (1985).
16. Narahashi, T. Effects of toxic agents on neural membranes. In: Electrophysiology in Neurotoxicology (H. E. Lowndes, Ed.), CRC Press, Boca Raton, FL, in press.
17. Lund, A. E., and Narahashi, T. Dose-dependent interaction of the pyrethroid isomers with sodium channels of squid axon membranes. Neurotoxicology 3: 11–24 (1982).
18. Wu, C. H., van den Bercken, J., and Narahashi, T. The structure-activity relationship of DDT analogs in crayfish giant axons. Pestic. Biochem. Physiol. 5: 142–149 (1975).